

Original Research

The impact of arbuscular mycorrhizal colonization on soil diversity indices of dill and common bean under different cropping systems

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ABSTRACT:

The purpose of this work was to compare the effects of *Funneliformis mosseae* and cropping system on soil bacterial and fungal diversity indices of dill (*Anethum graveolens* L.) and common bean (*Phaseolus vulgaris* L.), grown as the sole crop or intercropped in a field factorial experiment. The factors were cropping systems including a) common bean sole cropping (40 plants m⁻²), b) dill sole cropping at different densities (25, 50 and 75 plants m⁻²) and c) the additive intercropping of dill/common bean (25/40, 50/40 and 75/40 plants m⁻²). All these treatments were applied with or without Arbuscular Mycorrhiza (AM) colonization. The Shannone Wiener index and Evenness index of soil bacterial and fungal community were higher in the intercropping systems than those of sole cropping systems.

Keywords:

Anethum graveolens L., Bacterial and fungal community, Cropping system, *Funneliformis mosseae*.

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INTRODUCTION

Intercropping, the simultaneous cultivation of two or more crops in the same field, is practiced in many regions of the world. Depending on plant species, region and climate, intercropping can increase total yield per land area compared to sold cropping of the same crop. This is because intercropping makes better use of one or more agricultural resources in both space and time (Willey, 1990; Rodrigo *et al.*, 2001).

AM-induced changes in plant physiology affect the microbial populations, both quantitatively and qualitatively, in the rhizosphere and/or the rhizoplane. Therefore, the rhizosphere of a mycorrhizal plant can have features that differ from those of a non-mycorrhizal plant (Barea *et al.*, 2002; Johansson *et al.*, 2004). Soil microbial activity has been shown to depend on the presence of AM fungi and plant species (Wamberg *et al.*, 2003; Baligar *et al.*, 2005; Marschner and Timonen, 2006).

Bacterial community composition in the rhizosphere has also been shown to be affected by mycorrhizal colonization (Fillion *et al.*, 1999; Marschner and Baumann, 2003). However, little is known about the microbiological properties of the rhizosphere in intercropping systems. In intercropping, the roots of different plant species are in direct contact, and the rhizosphere communities of both crop types can therefore interact.

The resulting microbial community composition is likely to be a mixture of the species-specific communities, or may be dominated by the community composition of one plant species (Song *et al.*, 2007). To the best of our knowledge there are no reports on the

effect of AM fungi on the bacterial and fungal community in dill and common bean intercropping system. Thus, the objective of the present study was to evaluate the effects of mycorrhization by *Funneliformis mosseae* and cropping systems soil bacterial and fungal communities in rhizosphere of dill and common bean.

MATERIALS AND METHODS

Experimental Design

The field experiment was conducted in the Agriculture and Natural Resources Research Center of Kurdistan Province from April to early August 2014. Some soil physical and chemical properties were shown in Table 1. The experiment was carried out using a factorial arrangement based on randomized complete block design with three replications. The factors were cropping systems including: a) common bean (*Phaseolus vulgaris* L.) sole cropping (C40 = 40 plants m⁻²), b) dill (*Anethum graveolens* L.) sole cropping at different densities (D25, D50 and D75: 25, 50 and 75 plants m⁻², respectively) and c) the additive intercropping of dill/common bean (25/40, 50/40 and 75/40 plants m⁻²). All these treatments were applied with (+AM) or without (-AM) arbuscular mycorrhiza colonization. The crops were managed according to organic farming practices without pesticide or fertiliser use. No mechanical weeding was performed after sowing.

The mycorrhizal inoculum contained colonized root fragments, sand, AM hyphae, and spores. The inoculum was mixed with an inert material for dilution and homogenizing the distribution in the soil. All inocula were propagated as pure cultures on *Zea* maize L. for 6 months, in pots filled with an autoclaved (121 °C for 1 h

Table 1. Some physical and chemical properties of the soil of experimental area

Texture	Organic Carbon %	pH (1:2.5)	Electrical Conductivity (dSm ⁻¹) (1:2.5)	K	P	Ca	Na	Zn	Mn	Fe
(mg kg ⁻¹ soil)										
Sandy clay loam	1.14	7.12	0.072	131	12.2	1150.1	450.2	0.476	7.054	6.97

on three successive days) mixture of sand with 20% (v/v) field soil. A 30-g portion of inoculum was added to each plot (4m×5m) at sowing time just below the seeds. The plants were watered once a week to maintain soil moisture at about 50% of the water-holding capacity by adding tap water during the experimental period (Wang *et al.*, 2006). The arbuscular mycorrhiza fungi (*F. mosseae*) was obtained from the University of Tabriz, Iran.

Diversity indices

The diversity of soil bacterial and fungal community was calculated using the Shannon-Wiener index (H) and Evenness index (E). It was calculated as (Xu *et al.*, 2012):

$$H = \sum_{i=1}^S \left(\frac{N_i}{N} \right) \ln \left(\frac{N_i}{N} \right) \quad E_H = \frac{H}{\ln S}$$

Where N_i = was the number of the i th specie, N was the total number of species, S was the number of specie.

Arbuscular Mycorrhizal Colonization

Roots were prepared according to the modified method of Vierheilig *et al.* (2005). Small pieces of roots with 1 cm length were placed in a beaker (10% KOH) for

60 min within a water bath at 65° C. The root samples were then gently washed with tap water and acidified with 5% lactic acid for 12 h. A solution with 875 ml lactic acid, 63 ml glycerin, 63 ml tap water and 0.1 g fuchsin acid was used to stain roots for 30 min at 70° C. These roots were then de-stained by lactic acid for 15 min. Fungal root colonization was assessed by a Nikon Eclipse 80i light microscope with Nomarski interference contrast optics and a digital camera with a panel for image analysis. The percentage of mycorrhizal root colonization was calculated as (McGonigle *et al.*, 1990):
 %Root colonization = (number of root segments colonized/ number of root segments studied) × 100

Statistical Analysis

Combined analysis of variance was performed using SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) (SAS Institute Inc. 1988). Means of the treatments were compared, using Generalized Linear Model (GLM) method and the Least Significant Difference (LSD) test at the 5% probability level. The all data showed normal distribution and no transformation was required.

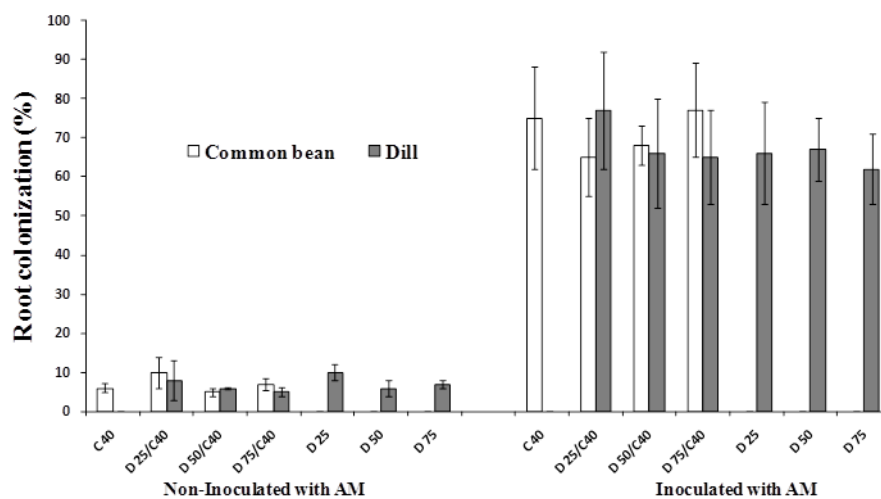


Figure 1. Root colonization percentage in common bean and dill under sole and intercropping systems inoculated and non-inoculated with arbuscular mycorrhizal fungus (*Funneliformis mosseae*). Means± S.D., $P < 0.05$.

C40: sole cropping of common bean (40 plants m^{-2}).

D25, D50 and D75: sole cropping of dill at 25, 50 and 75 plants m^{-2} , respectively.

D/C: dill/common bean intercropping.

Table 2. Wiener index (H) and Evenness index (E) of soil bacterial and fungal community of common bean and dill colonized (+AM) and not colonized with mycorrhiza (-AM) in sole and intercropping systems.

Treatments			Bacterial		Fungal	
			Shannone Wiener index (H)	Evenness index (E)	Shannone Wiener index (H)	Evenness index (E)
Intercropping	D75/C40	-AM	3.14±0.82	3.14±2.08	1.54±0.19	0.24±0.06
	D75/C40	+AM	2.99±1.16	2.98±3.22	1.11±0.05	0.12±0.01
	D50/C40	-AM	3.47±0.27	2.37±0.87	1.46±0.34	0.22±0.10
	D50/C40	+AM	3.51±0.15	1.68±1.08	1.05±0.17	0.11±0.04
	D25/C40	-AM	3.50±0.03	2.39±0.11	1.33±0.11	0.18±0.03
	D25/C40	+AM	3.66±0.01	1.08±0.05	1.14±0.01	0.13±0.00
Sole cropping	C40	-AM	3.54±0.14	1.53±1.06	0.89±0.08	0.08±0.01
	C40	+AM	3.48±0.07	0.65±0.10	1.23±0.02	0.15±0.01
	D75	-AM	3.38±0.37	0.83±0.65	1.11±0.20	0.13±0.05
	D75	+AM	3.50±0.13	0.97±0.67	1.06±0.17	0.11±0.04
	D50	-AM	3.64±0.04	1.05±0.16	1.15±0.03	0.13±0.01
	D50	+AM	3.64±0.05	1.03±0.19	1.15±0.04	0.13±0.01
	D25	-AM	3.66±0.02	1.13±0.17	1.13±0.03	0.13±0.01
	D25	+AM	3.60±0.03	0.89±0.07	1.18±0.01	0.14±0.00
Block			NS	NS	*	NS
CS			NS	*	*	*
AM			NS	NS	*	*
CS*AM			NS	NS	***	***

+ Comparison of means among cropping systems (CS) and mycorrhiza (AM) levels via *F* test (0.05).

Results are the mean of three replications ±SD.

NS, *, **, ***: Non-significant and significant at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively.

C40: sole cropping of common bean (40 plants m^{-2}).

D25, D50 and D75: sole cropping of dill at 25, 50 and 75 plants m^{-2} , respectively.

D/C: dill/common bean intercropping.

+AM, -AM: With and without mycorrhiza inoculation, respectively.

RESULTS AND DISCUSSION

AM Colonization

Arbuscular mycorrhizae was observed in all plant species and root samples (Fig 1). The percentage of mycorrhizal root colonization was significantly ($P \leq 0.01$) greater in all the mycorrhizal treatments than in the non-inoculated controls. There were no significant differences in colonization rates of sole and intercropping systems (Fig. 1).

Diversity indices

Shannon-Wiener index (H) and Evenness index (E) of soil bacterial and fungal community were higher under intercropping than under sole cropping systems. Soil fungal diversity indices were higher under AM

colonization and sole cropping (C40 and D75) than non-AM colonization and intercropping systems. However, in both sole and intercropping systems there was no significant difference between colonized and non-colonized plants in terms of soil bacterial Shannon-Wiener index (Table 2).

Our study showed that intercropping increased soil fungal and bacterial diversity in the rhizosphere (Table 2). This may be due to the interaction of soil microorganisms and different kinds of plant exudates, which may affect soil microbial communities (Zhou *et al.*, 2011). Plant species have previously been shown to differ in bacterial community composition in the rhizosphere. This may be explained by the fact that plant

species differ in exudate amount and composition (Merbach *et al.*, 1999), and microbial species differ in nutrient demand and capacity to decompose substrates.

Soil bacterial and fungal diversity was higher under intercropping (Table 2). Different field crops may or may not have differing effects on soil microbial community and diversity. However, soil microbial diversity does increase with increased above ground plant diversity (Garbeva *et al.*, 2006). Intercropping can increase microbial diversity when compared with crops grown in monoculture (Song *et al.*, 2007). The rhizosphere of monocropped wheat (*Triticum aestivum* L.) had more bacteria and fungi present than the rhizospheres of forage species such as ryegrass (*Lolium perenne* L.) or bent grass (*Agrostis capillaries* L.) (Grayston *et al.*, 1998). The differences in the microbial communities of various plant rhizospheres led to differences in carbon source utilization patterns, indicating that plant species affected microbial functional diversity (Grayston *et al.*, 1998).

CONCLUSION

Based on these results, it can be concluded that intercropping of dill and common bean, increased soil fungal and bacterial diversity in the rhizosphere. AM colonization of the main crops at different cropping systems improved Shannon-Wiener index and Evenness index of soil fungal community.

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